SOME EFFECTS OF OXYGEN UPON THE WHITE PUPAE OF HABROBRACON [1]

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Various investigations on a wide range of organisms have shown that high pressures of oxygen may have deleterious effects (Stadie, Riggs and Haugaard, 1944; Bean, 1945). Although injury from oxygen has been reported for insects the data have been rather meager. In 1878, Paul Bert in his studies on oxygen poisoning for a wide range of organisms reported that beetles are killed by high pressures of oxygen (Bean, 1945). A toxic effect from oxygen has been shown by Williams and Beecher (1944) for *Drosophila azteca* adults. Glass and Plaine (1952) reported a slight lag in development for *Drosophila melanogaster* exposed as embryos. Goldsmith and Schneiderman (1956) reported that various post-embryonic stages of *Mormoniella vitripennis* are sensitive to oxygen. Injurious effects from exposure to oxygen have been shown for *Habrobracon juglandis* where an arrestment of development, arrestment of pigmentation and a decrease in oxygen consumption occurs (Clark and Herr, 1954). The marked sensitivity of *Habrobracon* during certain stages of development indicated that this may be a good organism on which to study the mechanism of oxygen poisoning. In the present paper there are presented (1) data on the sensitivity of white pupae to oxygen, (2) data on the modification of oxygen-sensitivity by temperature.

Materials and Methods

The methods of rearing and of experimentation on *Habrobracon* have appeared in previous publications (Clark and Mitchell, 1951). Virgin females from Stock No. 33 were allowed to lay eggs during four hour periods. These eggs are haploid and accordingly develop into males. The cultures were allowed to develop for six days (approximately 144 hours). At this age all of the wasps are in the white pupal stage. Groups of pupae were placed into plastic chambers of about 100 cm.^{3} in volume. The chambers were flushed for one minute with 100 per cent oxygen delivered from a commercial cylinder and then exposed to oxygen at the desired pressure for one additional minute. The pupae were then removed from the plastic chamber into an air environment and observed for effects upon development and upon oxygen consumption. The eclosion ratio, the incidence of adults that emerge from cocoons, was used as a measure of survival. Groups of pupae that were exposed to 2 atmospheres of nitrogen showed no deleterious effects. This indicates that the injury reported here is not due to pressure.

Previous work has shown that the larval stages are resistant and the early pupal stages are quite sensitive to oxygen (Clark and Herr, 1954). White pupae (6-day

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cultures) appear to be most sensitive and were, therefore, selected for further experimentation. Pigmented pupae (7-day cultures), however, yield results comparable to those reported here for white pupae. The cultures were culled before use in order to remove individuals that were not in this stage, that had died, or that were too small. *Habrobracon* has a life cycle of 9 days at 30° C.

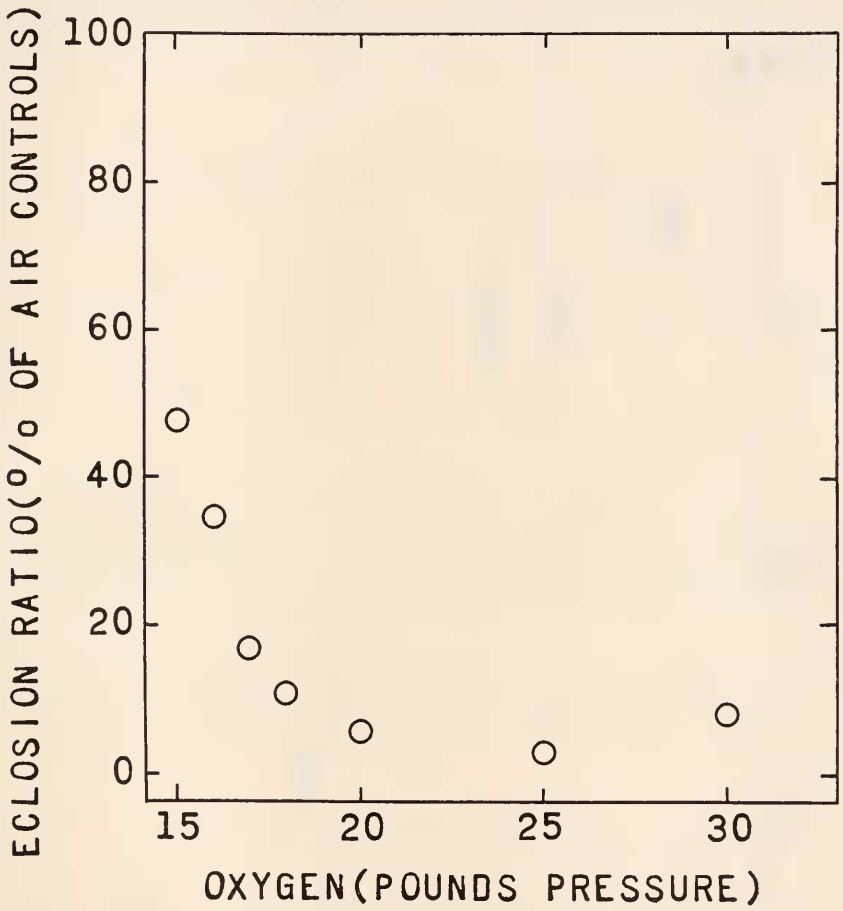

FIGURE 1. Eclosion ratios for wasps that were exposed to 100 per cent oxygen (15 to 30 pounds for one minute).

RESULTS

Groups of pupae were exposed to oxygen at pressures from 15 pounds to 30 pounds. A marked effect on the ability of the pupae to develop to the adult stage and to emerge from cocoons was observed. With increased pressures of oxygen from air to 20 pounds oxygen there is a decided decrease in the percentage of pupae that develop to the adult stage. After exposure to 20 pounds of oxygen only 5 per cent of the pupae develop to the adult stage (Fig. 1). The slight increase in eclosion for groups of pupae exposed to 30 pounds of oxygen is spurious since most

of the experiments at this dosage of oxygen yield zero eclosion. In fact, exposure of white pupae to 30 pounds of oxygen for only five seconds will arrest their development. Of 49 white pupae that were treated with 30 pounds of oxygen for 5 seconds, none developed to the adult stage.

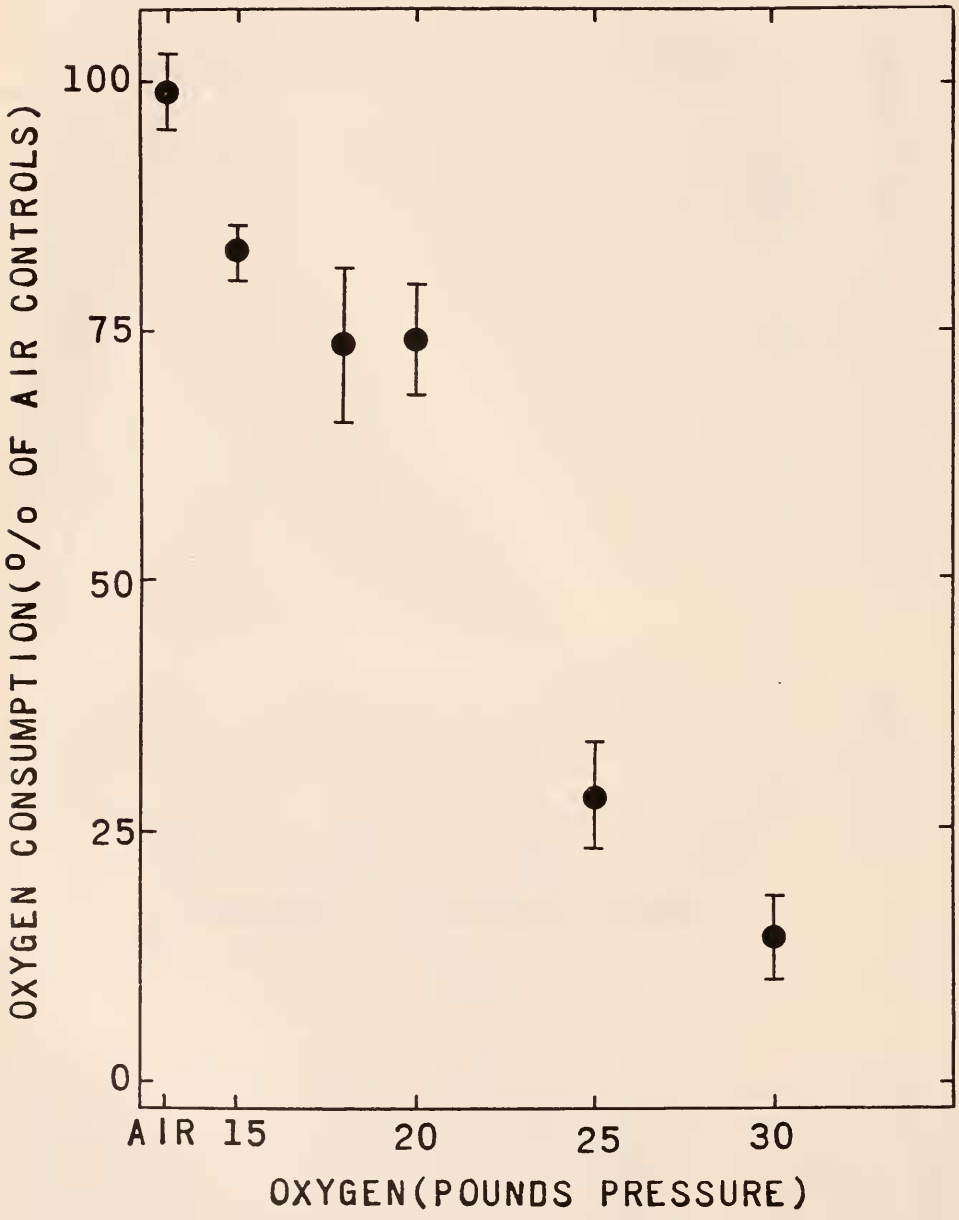

FIGURE 2. Oxygen consumption for white pupae that were exposed to 100 per cent oxygen (15 to 30 pounds for one minute).

White pupae treated with thirty pounds of oxygen are arrested at this stage of development. They remain as white pupae for about a week and after this time may become somewhat pigmented. They appear to be alive for at least two weeks as indicated by the lack of discoloration or the absence of drying-out of the pupae.

Further, such pupae showed the same magnitude of oxygen consumption after two days as after one hour. White pupae exposed to 15 pounds of oxygen become pigmented before they are arrested in development while white pupae treated with 30 pounds of oxygen are arrested immediately as white pupae. Within these

TABLE I

Respiratory quotients of white pupae exposed to oxygen

Treatment		Expressed as ul/25 pupae/hr.			
Gas	Pressure (pounds)	No. of experiments	O_2 consumed	CO_2 liberated	R. Q.
Air	15	6	40	27	.67
Oxygen	15	6	29	18	.61
Oxygen	20	6	15	10	.70
Oxygen	25	6	10	8	.77

extremes the amount of delay in pigmentation is influenced by the pressure of oxygen that is applied.

Groups of pupae (25 pupae/group) were treated with oxygen and then measured for oxygen consumption with a Warburg respirometer. The amount of oxygen utilized by the pupae was found to decrease as the oxygen pressure was increased (Fig. 2).

Comparison of the per cent of eclosion (Fig. 1) and the oxygen consumption (Fig. 2) shows that the degree of decrease is not of the same magnitude in each. The eclosion ratio drops off faster than the rate of oxygen uptake. After exposure

TABLE II

Oxygen uptake for white pupae exposed to oxygen
(measured 1 and 24 hours after treatment)

Treatment		ul O_2/25 pupae/hr.	
Gas	Pressure (pounds)	1 hour	24 hours
Air	15	38	32
O_2	15	28	26
O_2	20	11	12
O_2	25	8	8

to an oxygen pressure of 20 pounds the eclosion falls to about 5 per cent of the air controls while for the oxygen uptake it falls to about 75 per cent of the air controls. Thus, the decrease in the eclosion percentage is most marked in the oxygen dosage range from air to 20 pounds of oxygen, while the greatest decrease in oxygen consumption does not occur until after treatment within the dosage range of 20 to 30 pounds of oxygen. The data indicate that there is no difference in oxygen uptake between pupae treated with 18 pounds or with 20 pounds of oxygen (Fig. 2).

The respiratory quotient (R. Q.) was determined after exposure to various pres-

TABLE III

Eclosion ratios of white pupae exposed to oxygen at 10° C. and 26° C.

Treatment		Eclosion ratio	
Gas	Pressure (pounds)	26° C.	10° C.
Air	15	74–82	41–47
O_2	15	12–58	48–57
O_2	18	7–41	47–55
O_2	20	5–101	79–93
O_2	25	0–36	34–50

sures of oxygen (Table I). In these experiments groups of pupae were measured for oxygen consumption for three hours, after which the KOH was removed from the center well and the amount of carbon dioxide liberated was determined. With increasing pressures of oxygen there is a decrease both in the oxygen consumption and in the liberation of carbon dioxide. There is no change in the R. Q. with increased oxygen pressure (Table I).

It seemed of interest to inquire whether there was any recovery of the ability to consume oxygen following treatment with oxygen. In order to test this, pupae whose oxygen consumption had been measured within one hour after treatment with oxygen pressures from 15 to 25 pounds were kept in an incubator for 24

TABLE IV

Oxygen uptake of white pupae exposed to oxygen at 10° C. and 26° C.

Treatment		ul O_2/25 pupae/hr.	
Gas	Pressure (pounds)	26° C.	10° C.
Air	15	44	46
O_2	25	21	47

hours and then re-measured for oxygen consumption. These data appear in Table II and show that there is no change in oxygen uptake after 24 hours. Experiments not reported here have shown that the oxygen consumption does not increase after two days. Thus, the decrease in oxygen consumption following oxygen treatment is irreversible.

Groups of white pupae were placed into a refrigerator at 10° C. for ½ hour or kept at room temperature (26° C.) for the same length of time. They were then exposed immediately to oxygen of known pressure and placed into the incubator (30° C.) to observe for developmental effects. Pupae treated when cold were much more resistant to oxygen than were the pupae that were treated when warm (Table III). For example, of 50 pupae that were treated with 25 pounds of oxy-

TABLE V

Eclosion ratios of white pupae treated with oxygen before and after exposure to 10° C. and 26° C.

	Eclosion ratio Oxygen pressure (pounds)		
Treatment	15	20	25
10° C., then O_2, then 10° C.	91–106	62–72	26–43
26° C., then O_2, then 10° C.	34–90	1–58	0–30
10° C., then O_2, then 26° C.	84–96	34–44	33–41
26° C., then O_2, then 26° C.	35–76	3–42	0–35

gen after exposure to 10° C., 35 developed to the adult stage and emerged from their cocoons while of 36 pupae treated in the same manner after exposure to 26° C., none developed to the adult stage and eclosed. Other groups of pupae were treated in the same manner and measured for oxygen uptake. Pupae exposed to 25 pounds of oxygen after cold exposure consumed as much oxygen as the controls while pupae treated after exposure to warm temperature showed a marked decrease in oxygen consumption (Table IV). These data on oxygen consumption are in agreement with the data on eclosion (Table III).

Since temperature has an effect on the sensitivity of pupae to oxygen, the possibility that this oxygen-sensitivity could be modified by exposure to different temperatures after oxygen treatment was considered. Groups of white pupae were placed either at 10° C. or 26° C. for ½ hour, then exposed to oxygen of known pressure and then placed at 10° C. or at 26° C. for one hour (Table V). Eclosion ratios were obtained from the pupae so treated and showed that the post-treatment with temperature had no effect upon recovery. Thus, the temperature at the time of treatment with oxygen modified the oxygen-sensitivity. Whether longer periods of post-treatment with temperatures of 10° C. would be effective has not been tried. The metabolic state of the organism at the time of treatment seems, therefore, to determine the extent of its sensitivity.

Discussion

Habrobracon white pupae when exposed to oxygen show an immediate and marked decrease in oxygen consumption and, subsequently, an arrestment of development and of pigmentation. The magnitude of these effects can be correlated to a certain degree with the dosage of oxygen that is applied to these organisms.

It seems clear that the arrestment of pigmentation is due to the lack of sufficient oxygen in the tissues to allow for the enzymatic oxidation of tyrosine to melanin. This seems to be indicated by the following observations. The steep drop in the pigment-forming ability occurs after those dosages of oxygen where a marked decrease in oxygen consumption occurs (between 20–30 pounds pressure, Figure 2). At doses of less than 20 pounds of oxygen, there is relatively little decrease in pigmentation and in oxygen consumption. The arrestment of pigmentation in *Habrobracon* white pupae can be brought about also by exposure of the pupae to lowered concentrations of oxygen. There is no recovery in the rate of oxygen consumption for pupae whose oxygen consumption has been lowered by exposure to oxygen. It is generally realized that an arrestment of pigmentation may be caused by exposure of insects to environments with less oxygen tension. The fact that arrestment of pigmentation may be caused by increased oxygen pressures was reported by Linden in 1906 (Sussman, 1949).

The events that are responsible for the arrestment of development may be different from those responsible for the arrestment of pigmentation since pupae that are exposed to 15 pounds of oxygen show an arrested development but exhibit no delay in the acquiring of pigment. It seems difficult to relate this arrested development to a decrease in available oxygen since the incidence of pupae that develop to the adult stage after exposure to 20 pounds of oxygen is low (5 per cent of controls) while their rate of oxygen consumption is relatively high (75 per cent of controls). It is possible that the arrested development may be due to the inac-

tivation of a substance that has some control over development or to an increased concentration of some toxic materials.

Various authors (see Bean, 1945) have suggested that the primary effect of exposure to oxygen gas is the inactivation of oxidative enzymes with a resultant generalized tissue anoxia. The fact that there is an immediate decrease in oxygen consumption for *Habrobracon* pupae indicates that this hypothesis may be valid. Studies on the oxygen uptake and enzyme activity of tissue homogenates, at present in progress, are needed to show this. To date, however, no decrease in oxygen consumption or in succinic dehydrogenase activity of homogenates from oxygen-treated pupae has been observed. Extensive experiments bearing on this hypothesis of tissue anoxia have been carried out by Stadie, Riggs and Haugaard (1944) with negative results. They found no immediate reduction in oxygen uptake in tissues from rats that had been killed by 7 atmospheres of oxygen. They assume, therefore, that generalized tissue anoxia is not the cause of acute oxygen poisoning. Despite this, it is not possible to eliminate the possibility that localized tissue anoxia may occur.

All stages of development in *Habrobracon* are not equally sensitive to the injurious effects of oxygen (Clark and Herr, 1954). The larval and prepupal stages are not affected by 30 pounds of oxygen, while almost all of the pupae are injured. The reason for this difference in stage-sensitivity is not known at present. It seems clear, however, that it is not due simply to a difference in the rate of metabolism. Based upon oxygen consumption studies one can show that the oxygen-resistant larvae are more active than are the oxygen-sensitive pupae. In the present paper, however, experiments have been given that show that pupae that have been made less active by exposure to a temperature of 10° C. are more resistant to the toxic effects of oxygen than are pupae that were kept at 26° C. immediately before treatment. It seems that some qualitative difference in the metabolism of larvae and pupae exists that can be related to this difference in sensitivity. Our primary aim, then, is to determine the nature of these differences during development.

The marked and immediate decrease in oxygen consumption for *Habrobracon* pupae and the absence of a compensating recovery is surprising. In the wasp *Mormoniella vitripennis,* exposure of black pupae to 5 atmospheres of oxygen for from 4 to 6 hours prevented 50 per cent from emerging but their oxygen uptake was unimpaired (Goldsmith and Schneiderman, 1956). We have treated pupae of other insect species with 2 atmospheres of oxygen under conditions comparable to those that we used for *Habrobracon* but no obvious effects on oxygen uptake or on development have been observed. The species tested were *Drosophila melanogaster, Musca domestica, Ephestia kuhniella* and *Polistes* sp. It is hard to imagine that other species of insects do not exist that exhibit strong oxygen-sensitivity and, therefore, our search for other insects in this category continues.

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Summary

1. *Habrobracon* were exposed as white pupae to oxygen and studied for effects upon development, oxygen consumption and pigmentation.

2. A marked decrease in the incidence of pupae that complete development occurs after exposure to oxygen within the range from air to 20 pounds. The greatest decrease in the rate of oxygen uptake and pigmentation occurs after exposure within the range from 20 to 30 pounds.

3. The decrease in oxygen uptake following treatment is immediate. No subsequent recovery of oxygen uptake was observed 24 hours after treatment.

4. There is no modification of the respiratory quotient following treatment with oxygen. With increasing pressures of oxygen both the oxygen consumption and carbon dioxide liberation decrease at the same rate.

5. The sensitivity of white pupae to oxygen is modified by temperature. Pupae treated when cold are more resistant than pupae treated when warm. Thus, lowering the metabolic state of the pupae increases their resistance to oxygen.

6. The inability of the oxygen-treated pupae to acquire pigmentation has been explained on the basis of insufficient oxygen to allow for the oxidation of tyrosine to melanin. The effect of the oxygen treatment upon oxygen consumption and on development is unexplained and at present obscure.

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